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Effects of plant species diversity and composition on nitrogen cycling and the trace gas balance of soils

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Abstract

Experiments addressing the role of plant species diversity for ecosystem functioning have recently proliferated. Most studies have focused on plant biomass responses. However, microbial processes involved in the production of N_2O and the oxidation of atmospheric CH_4 could potentially be affected via effects on N cycling, on soil diffusive properties (due to changes in water relations and root architecture) and by more direct interactions of plants with soil microbes. We studied ecosystem-level CH_4 and N_2O fluxes in experimental communities assembled from two pasture soils and from combinations of 1, 3, 6, 8 or 9 species typical for these pastures. The soils contrasted with respect to texture and fertility. N_2O emissions decreased with diversity and increased in the presence of legumes. Soils were sinks for CH_4 at all times; legume monocultures were a smaller sink for atmospheric CH_4 than non-legume monocultures, but no effect of species richness *per se* was detected. However, both the exchange of CH_4 and N_2O strongly depended on plant community composition, and on the interaction of composition with soil type, indicating that the functional role of species and their interactions differed between soils. N_2O fluxes were mainly driven by effects on soil nitrate and on nitrification while soil moisture had less of an effect. Soil microbial C and N and N mineralisation rates were not altered. The driver of the interactive soil type×plant community composition-effects was less clear. Because soil methanotrophs may take longer to respond to alterations of N cycling than the 1/2 year treatment in this study, we also tested species richness-effects in two separate 5-year field studies, but results were ambiguous, indicating complex interactions with soil disturbance. In conclusion, our study demonstrates that plant community composition can affect the soil trace gas balance, whereas plant species richness *per se* was less important; it also indicates a potential link between the botanical composition of plant communities and global warming.

Introduction

The role of plant species diversity and community composition in ecosystem performance has long been of interest to ecologists (Elton, 1958; MacArthur, 1955). Despite this, it is still often not possible to predict the functioning of even quite simple plant assemblages from the traits of the component species (Loreau, 2000; Lavorel and Garnier, 2002; Wardle et al., 1999). Experiments addressing this question, and hypotheses about the underlying mechanisms, have as

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consequence proliferated (Schläpfer and Schmid, 1999). While experimental biodiversity studies have mostly been focused on ecosystem functions related to plant biomass and the carbon cycle (e.g., biomass, photosynthesis, leaf area, litter decomposition), effects on the trace gas balance have not been considered so far.

Methane (CH_4) and nitrous oxide (N_2O) currently contribute ≈20% to anthropogenic radiative forcing and also affect atmospheric chemistry (IPCC, 2001). N_2O is produced by soil microbes both during nitrification and denitrification (Conrad, 1996; Schlesinger, 1996). Nitrification is the main source of N_2O under aerobic conditions while denitrification dominates under anoxic conditions. Denitrification is highest when NO_3^- is available and organic C is present as a reductant (Firestone and Davidson, 1989; Paul and Clark, 1996; Robertson and Tiedje, 1987). CH_4 is both produced and consumed by soil microbes and accordingly, soils can be sources and sinks of atmospheric CH_4. Methanogenesis dominates in wetlands, while CH_4 oxidation, a process primarily carried out by aerobe, obligate methanotrophic bacteria, often dominates in well aerated soils (Hanson and Hanson, 1996; Schlesinger, 1996). Soil methanotrophy is the only biological sink for atmospheric CH_4 and therefore has a key place in the global CH_4 cycle.

Little is known about how plant diversity and composition affect ecosystem-level N_2O and CH_4 exchange. However, we propose that there are several mechanisms by which plant diversity may affect the exchange of these gases.

Highly diverse plant communities will more fully capture available mineral N (e.g., Ewel et al., 1991; Niklaus et al., 2001a; Spehn et al., 2005). A suggested mechanism is interspecific differences in resource use (McKane et al., 2002). Conversely, a lack of complementarity among species in space (root architecture), time (phenology) or mode of N uptake (chemical form of N, root uptake kinetics) will increase NH_4^+ and NO_3^- concentrations. Higher nitrification rates due to higher [NH_4^+] and increased denitrification rates due to higher [NO_3^-] will both increase N_2O emissions.

Legumes play a key role by introducing atmospheric dinitrogen into biotic pools and their presence often affects the performance of the whole community (e.g., Craine et al., 2002; Spehn et al., 2002). Typically, legumes reduce soil N uptake by plants and lead to higher soil inorganic N. This accumulation of inorganic N in soils can result in increased nitrification and denitrification rates and thereby increased N_2O emissions. In mixed legume–non-legume communities, non-legumes may partly compensate for legume-effects on soil NO_3^- by reducing the soil inorganic N pool (Hooper and Vitousek, 1997).

Plant diversity also may alter soil CH_4 exchange. CH_4 oxidation has been shown to be inhibited by NH_4^+, either due to substrate competition at the enzymatic level (NH_4^+ and CH_4 are isoelectronic and appear both to bind to the key enzyme with which methanotrophs assimilate CH_4 [methane mono-oxygenase]; Dunfield and Knowles, 1995) or because of more complex competitive interactions between nitrifiers and methanotrophs (Powlson et al., 1997). However, the ecology of CH_4 oxidisers in oxic soils is far from being well understood. Increased CH_4 oxidation at increased soil NH_4^+ concentratios has also been reported (Bodelier and Laanbroek, 2004), challenging the concept of inhibition by NH_4^+ as a main regulatory factor of CH_4 oxidation in soils. Nevertheless, N cycling processes appear to affect the activity of methanotrophs, either negatively or positively, depending on conditions (Bodelier and Laanbroek, 2004).

Another major control of soil trace gas fluxes is soil moisture. Nitrification rates are relatively insensitive to changes in soil moisture but denitrification increases dramatically when anaerobic microsites increase. At the same time, higher soil moisture has been found to limit methanotrophy by slowing diffusion of CH_4 and O_2 (Del Grosso et al., 2000; Smith et al., 2000). While plant diversity could have an effect on soil moisture because plant communities might differ in water consumption, this has rarely been found in plant diversity experiments (e.g., Spehn et al., 2000a), presumably because water use is largely driven by radiative energy budgets and not so much by leaf area and phenology.

Finally, plant diversity might also affect trace gas exchange more indirectly, for example by altering the mineralisation of organic matter (by changing litter quality and by priming effects on decomposition), by affecting N immobilisation in soil microbial biomass, and by changing C availability to denitrifiers and soil microbial communities in general.

A mechanistic understanding of how plant species affects soil trace gas dynamics is essential to understand potential links between biodiversity and greenhouse warming. In the present paper, we test whether plant community composition and diversity affect the soil N_2O and CH_4 balance, contributing to closing this gap in knowledge. We also test whether previously observed patterns of diversity-ecosystem function relationships will extended to trace gas fluxes.

Materials and methods

Design and setup of main experiment

We set up an experiment consisting of 128 microcosms arranged in a randomised block design with soil type and plant diversity as factorial treatments. The soil treatment consisted of soils from a low- and a moderately fertile sheep-grazed pasture (hereafter named *Ballantrae* and *Flockhouse* after their origin). The plant diversity treatment consisted of plant communities, which were assembled from a common species pool so that their species and functional type number varied (Table 1).

The soils were sampled from two locations in the south–west of New Zealand's north island. The low-fertility soil was collected on a hill-country sheep pasture at *Ballantrae* experimental station (AgResearch, New Zealand; Saggar et al., 2000). It is classified as a Typic Dystrudept (USDA 1999) and the top soil has a fine loamy texture and a pH of 5.6. The more fertile soil was sampled in a lowland sheep-grazed pasture at *Flockhouse* (Edwards et al., 2001). This Mollic Psammaquent has a dark, loamy fine sand top soil horizon of 25 cm, organic matter content of 5%, pH 6.6 and is hydrophobic. At both field sites, 15 cm of top soil were collected, roots and plant fragments removed, and the soils passed through a coarse sieve (8 mm) and thoroughly mixed. Microcosms with a volume of 6.3 L each were prepared by filling the bottom with 4 cm of gravel and covering it with a fine nylon cloth (2 mm mesh size). These microcosms were then filled with the soils from the respective sites and watered twice to let the soils consolidate to about their field bulk density.

Three legume, three grass, and three non-legume forb species were raised from seed before they were transplanted to microcosms. These plant species were typical of the sheep pastures from which the soils had been taken. Plant diversity treatments consisted of monocultures, 3-species mixtures with either one or all three functional types, 6-species mixtures with 2 or 3 functional groups, 8-species mixtures containing all but one species, and mixtures were all 9 species were grown together (Table 1). The species composition of pairs of monocultures and 8-cultures as well as of pairs of

Table 1. Composition of experimental communities

Species composition (legumes–grasses–forbs)[a]					
NIL			9 species, 3 FG		
			ABC-abc-$\alpha\beta\gamma$		
1 species, 1 FG			8 species, 3 FG		
A	B	C	BC-abc-$\alpha\beta\gamma$	AC-abc-$\alpha\beta\gamma$	AB-abc-$\alpha\beta\gamma$
a	b	c	ABC-bc-$\alpha\beta\gamma$	ABC-ac-$\alpha\beta\gamma$	ABC-ab-$\alpha\beta\gamma$
α	β	γ	ABC-abc-$\beta\gamma$	ABC-abc-$\alpha\gamma$	ABC-abc-$\alpha\beta$
3 species, 1 FG			6 species, 2 FG		
ABC	abc	$\alpha\beta\gamma$	abc-$\alpha\beta\gamma$	ABC-$\alpha\beta\gamma$	ABC-abc
3 species, 3 FG			6 species, 3 FG		
Aaα	Bbβ	Ccγ	bc-BC-$\beta\gamma$	AC-ac-$\alpha\gamma$	BC-bc-$\beta\gamma$

Communities listed in the left and right columns are complements, species-wise, e.g., Aaα and BC-bc-$\beta\gamma$ added together give the full species set ABC-abc-$\alpha\beta\gamma$.

[a] A: *Trifolium subterraneum* L., B: *T. repens* L., C: *T. pratense* L., a: *Lolium perenne* L., b: *Anthoxanthum odoratum* L., c: *Agrostis capillaris* L., α: *Plantago lanceolata* L., β: *Leontodon saxatilis* Lam., γ: *Hypochaeris radicata* L.

3-cultures and 6-cultures were complementary, i.e., when their compositions were added, all 9 species were present in equal proportions (Table 1). This design is an extension of the one used by Wardle et al. (2003) and allows to test the effect of the presence and absence of individual species and species groups, and also equalises the abundance of species across all richness levels; this avoids some problems associated with the 'sampling effect' which has been identified as a hidden treatment in other studies (cf. Huston, 1997). There were four microcosms per species combination (2 replicates on each soil) except for the bare soil and the 9 species microcosms were there were twice as many replicates. Plantlets were arranged on a hexagonal grid with 35 mm spacings (36 individuals per microcosm); the positions of the individual species on this grid were determined randomly. Each randomisation was then assigned to a pair of microcosm with *Ballantrae* and *Flockhouse* soils, i.e., there were two randomisations per soil type for the 3, 6, and 8 species mixtures and four randomisations for the 9 species mixtures.

Microcosms were placed in two separate greenhouses; these two replicate blocks of the experiment utilised independently randomised species positions for any given community composition. Planting occurred in October 2001; 2 weeks later, plants were clipped to a height of 3 cm and the experiment started. There was no mortality of transplanted individuals and weeds were removed regularly so that the composition of microcosms remained constant throughout the study. Plants were subject to regular watering but no fertiliser was added throughout the experiment.

Plant material

Plants were clipped to a height of 3 cm above ground in mid-December (early summer), at the end of January (mid summer), in early March (early autumn), and finally clipped down to ground level in early May (autumn) when the experiment was terminated. At the final harvest, roots were recovered by floatation and wet-sieving over a 0.5 mm sieve. All plant material was dried overnight at 80 °C, weighed, ground and analysed for C and N contents using an automated CHN analyser (Model FP 2000, LECO Corp., St Joseph, MI, USA).

Microbial biomass

Soil microbial biomass C and N were determined by chloroform fumigation-extraction (Brookes et al., 1985; Vance et al., 1987). Soils were sieved (2 mm mesh size), homogenised, and remaining roots carefully removed with tweezers. One of two field-moist subsamples (water content of 40–50% water capacity, weight corresponding to 10 g dry weight) was immediately extracted for 30 min with 100 mL 0.5 M K_2SO_4 on an end-over shaker. The second sample was fumigated with ethanol-free $CHCl_3$ for 24 h and $CHCl_3$ removed by repeated evacuation. Fumigated samples were then extracted in the same way as the unfumigated soils. All extracts were centrifuged (10 min at 1000 *g*), filtered (Advantec 5C acid-washed filter paper, Toyo Roshi Kaisha Ltd., Japan) and kept frozen until further analysis. Organic C in the extracts was determined using an automated TOC analyser (High TOC II, Elementaranalysensysteme GmbH, Hanau, Germany); organic N was measured by alkaline persulphate oxidation followed by measurement of NO_3^- in the extracts (Lachat Quikchem 8000 flow injection analyser; Zellweger Analytics, Milwaukee, WI, USA). Microbial biomass C was calculated as $C_{mic} = (C_{fumigated} - C_{unfumigated})/k_{EC}$, where $k_{EC} = 0.41$ is the extraction efficiency for microbial C and $C_{fumigated}$ and $C_{unfumigated}$ are the organic C contents of the extracts of fumigated and unfumigated soils, respectively. Microbial biomass N was similarly calculated using $k_{EN} = 0.54$.

Soil ammonium and nitrate

Before the final plant harvest, three soil cores (1.5 cm diameter × 10 cm depth) were taken per microcosm, extracted with 100 mL 2 M KCl, and analysed for NH_4^+ and NO_3^- (Lachat Quickchem FIA 8000, Zellweger Analytics, Milwaukee, WI, USA).

Nitrogen mineralisation

Net nitrogen mineralisation was determined in laboratory incubations both under aerobic and anaerobic conditions. For aerobic N mineralisation, soil samples (10 g fresh weight each) were

incubated at 25 °C and 60% of WHC in 125 mL containers. These were enclosed in trays containing water to prevent drying of the samples. NH_4^+–N and NO_3^-–N in samples incubated for 0, 7, 14, 28 and 56 days were extracted with 100 mL 2 M KCl and determined colourimetrically (Lachat Quickchem FIA 8000). Anaerobic N mineralisation was determined in soil samples (10 g fresh weight), which were incubated for 7 days at 40 °C in glass vials that contained 10 mL of extra water (this resulted in minimal head space; see also Niklaus et al., 2001a). Inorganic N produced was determined as above.

Potential nitrification

Potential nitrification rates of soils was determined as short-term NO_3^- production during incubation in a KH_2PO_4/K_2HPO_4 buffer (pH 7.0) containing excess NH_4^+ (as $(NH_4)_2SO_4$) (Schmidt and Belser, 1994). Briefly, 20 g of fresh soil were added to 100 mL buffer solution and incubated at 25 °C in Erlenmeyer flasks on a table shaker. After 1, 4, 8 and 23 h, 10 mL samples of the suspension were removed, amended with 5 mL 2 M KCl to stop nitrification, the sample centrifuged, the supernatant filtered and analysed for NO_3^- concentrations. Potential nitrification rates were calculated as the NO_3^- concentration increase per gram soil per hour.

Soil CH_4 and N_2O exchange

After each clipping of plants, CH_4 and N_2O exchange of microcosms were measured. The entire microcosms were placed in completely closed static chambers (13.7 L volumes) that were equipped with a water-filled pressure equalisation siphon (CH_4 is practically insoluble in water, and N_2O dissolution was too slow to be detected during the incubation). Gas samples taken after 0, 1 and 2 h were analysed for CH_4 and N_2O concentrations using a gas chromatograph (Shimadzu GC17A; Shimadzu Corp., Columbia, Maryland, USA) fitted with a flame ionisation (FID) and a ^{63}Ni-electron capture detector (ECD). Gas exchange rates were calculated by linear regression of CH_4 and N_2O concentration against time; r^2 was >0.99 except for very low gas exchange rates. Concomitantly, soil moisture was measured with a frequency-domain reflectometry probe (Thetaprobe ML2, Delta-T Devices, Burwell, Cambridge, UK).

CH_4 oxidation in long-term field studies

To complement the short-term (6 month) data gained from the experiment described above, soil samples from two additional longer-term field biodiversity studies were collected: The first study (Niklaus et al., 2001b) consisted of calcareous grassland communities with 5, 12 and 31 species, combined with a factorial elevated CO_2 treatment. The second study consisted of the Swiss site of a pan-European biodiversity experiment in which plant communities were randomly selected from a species pool of 48 species to create cultures with 1, 2, 4, 8, 16 and 32 species (Hector et al., 1999; Spehn et al., 2002); this study contained a factorial soil disturbance treatment mimicking animal hoof impacts (cf. Pfisterer and Schmid, 2002).

Soil samples from both experiments were coarsely sieved and incubated in gastight jars; head space CH_4 concentration was brought to 5 ppmv and the depletion of the head space CH_4 followed by repeated sampling and GC analysis. First-order rate constants for CH_4 oxidation were calculated by non-linear regression.

Data analysis

All data were analysed by analysis of variance using R 1.9 (R Development Core Team, 2004). Factors were fitted sequentially (type I sum of squares) as indicated in Table 2. The structure of the model followed generally design principles that have been applied in many other diversity studies (e.g., Bartelt-Ryser et al., 2005; Hector et al., 1999; Spehn et al., 2000b) – Schmid et al. (2002) provide a detailed discussion of the foundations of this approach. We first fitted a factor *soil cover* distinguishing between bare soil microcosms and vegetated microcosms (which all had close to 100% soil cover). Effects of diversity were then tested within the vegetated systems only; diversity tests were split into species and functional-type richness components, and each of these tests was further split into a test for linear dependence on diversity and a test for deviation from linearity.

Table 2. Skeleton analysis of variance

Source of variation	Nominator df	Denominator	
		Term	df
Intercept	1		
Block	1		
Soil	1	Residual	71/65/65
Cover	1/–/–	Residual	24/–/–
spdiv	4		
spdiv{log}	1	Mixture	24/24/23
spdiv{deviation}	3	Mixture	24/24/23
fgdiv	2		
fgdiv{linear}	1	Mixture	24/24/23
fgdiv{nominal}	1	Mixture	24/24/23
sp-contrast	–/–/1	Mixture	–/–/24
Soil×Cover	1/–/–	Soil×Mixture	24/–/–
Soil×spdiv	4		
Soil×spdiv{log}	1	Soil×Mixture	24/24/23
Soil×spdiv{nominal}	3	Soil×Mixture	24/24/23
Soil×fgdiv	2		
Soil×fgdiv{linear}	1	Soil×Mixture	24/24/23
Soil×fgdiv{nominal}	1	Soil×Mixture	24/24/23
Soil×sp-contrast	–/–/1	Soil×Mixture	
Mixture	24/24/23	Residual	71/65/65
Soil×mixture	24/24/23	Residual	71/65/65
Residual	71/65/65		
Total	136/128/128		

Three different models were used to test for effects of plant cover (model 1 included the additional terms *cover* and *cover×soil*), plant diversity (model 2), and the effect of the presence of single species or functional types (model 3 included *sp-contrast* and *soil×sp-contrast*). Model 2 and 3 were run under exclusion of bare soil microcosm. For model 2, the order of terms is shown for tests of species richness; effects of functional type richness were tested by reversing the order of spdiv and fgdiv and of their interactions. Degrees of freedom for model 1, 2 and 3 are given (in this order) wherever they differ.

For example, we first fit log(species richness) to test for a log-linear dependency of our variables on species richness, and then fitted species richness as nominal factor to test for deviation from this log-linear relationship. Similarly, functional type number was fitted first as a linear and then as nominal factor.

All data were log-transformed prior to analysis to achieve normal distribution and in order for interactions to test for equality of relative treatment effects. All data indicated in tables and figures are means ± s.e. Stepwise multiple regression maximising Akaike's information criterion was used to analyse the dependency of trace gas exchange on soil moisture, soil NO_3^-, and potential nitrification rates (stepAIC procedure in R; Venables and Ripley, 2002). Gas exchange rates (CH_4 and N_2O), soil NO_3^- and potential nitrification rates were log-transformed for this analysis.

Results

Plant nitrogen productivity

Plant nitrogen productivity (g N m^{-2} in clipped biomass; Figure 1) was higher on the more productive Flockhouse soil ($P < 0.001$) and increased with species richness on both soils ($P < 0.01$). Legumes massively increased community productivity: legume-only communities had almost twice the plant N productivity compared to non-legumes communities ($P < 0.001$); monocultures and three-species mixtures consisting of legumes only exhibited N productivities as high (Ballantrae) or higher (Flockhouse) than the most species-rich communities. Belowground effects of species richness and legume presence qualitatively corresponded to the effects found above-ground but were smaller.

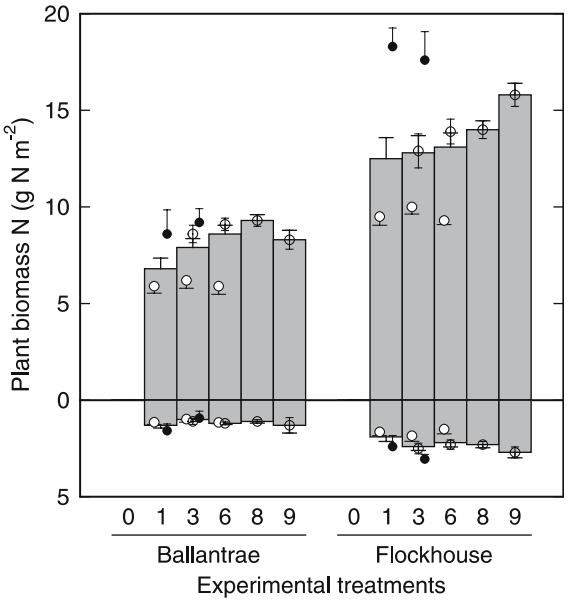

Figure 1. Plant community nitrogen productivity as a function of plant species diversity (1, 3, 6, 8 or 9 species) and soil type; above-ground N is the sum of clippings above 3 cm (first three clippings) plus total above-ground biomass N at the destructive harvest. Belowground biomass N is total root N at the final harvest. Bars indicate averages of communities with a given species richness (means ± s.e.); black dots with upward error bars indicate values for communities containing legumes only (upward error bars only); white dots indicate values for communities without legumes (downward error bars only); open circles with up and downward error bars indicate communities which contain both legumes and non-legume species.

Table 3. Soil moisture (average over all dates on which gas exchange measurements were conducted) and microbial biomass C and N as a function of plant species richness and soil type (means s.e.)

Soil	Species richness	Soil moisture g cm^{-3}	Microbial C μg C (g soil)$^{-1}$	Microbial N richness μg N (g soil)$^{-1}$	Microbial C/N μg C (μg N)$^{-1}$
Ballantrae	0	0.29 ± 0.01	323 ± 42	58.5 ± 8.3	5.6 ± 0.6
	1	0.28 ± 0.01	383 ± 11	62.1 ± 1.8	6.2 ± 0.2
	3	0.27 ± 0.02	362 ± 9	66.3 ± 1.5	5.5 ± 0.1
	6	0.23 ± 0.01	363 ± 15	70.2 ± 4.7	5.3 ± 0.3
	8	0.25 ± 0.01	333 ± 12	64.1 ± 1.3	5.2 ± 0.2
	9	0.25 ± 0.02	367 ± 23	73.6 ± 7.9	5.1 ± 0.5
Flockhouse	0	0.39 ± 0.01	345 ± 97	30.4 ± 2.2	11.3 ± 2.7
	1	0.28 ± 0.02	282 ± 21	37.1 ± 2.2	7.7 ± 0.4
	3	0.26 ± 0.02	277 ± 21	41.4 ± 3.3	6.8 ± 0.4
	6	0.24 ± 0.02	258 ± 19	38.3 ± 7.0	8.4 ± 1.2
	8	0.22 ± 0.01	271 ± 15	45.7 ± 3.7	6.5 ± 0.7
	9	0.27 ± 0.01	267 ± 29	42.7 ± 5.8	6.8 ± 1.6

Soil microbial biomass

Soil microbial biomass C was higher in the finer-textured Ballantrae soil than in Flockhouse soil. Microbial C did not respond to the presence of plants, to the diversity of plant communities or to the presence of individual species (Table 3). Microbial N by contrast was higher with plant cover ($P < 0.05$) and increased marginally significantly with plant species diversity ($P = 0.06$) and number of plant functional types ($P = 0.07$). Neither legume presence nor the presence of any other functional type or species affected microbial N. When microbial N was expressed relative

to C, effects of plant cover and diversity were highly significant ($P < 0.001$) and legumes tended to decrease microbial C/N.

Soil moisture

Soil moisture varied from 20% to 31% (weight/volume), depending on date and soil, and was generally higher in bare soil microcosms (Tables 3 and 4). There was a slight tendency for soil moisture to decrease at higher diversity.

Soil ammonium and nitrate

Soil [NH_4^+] (Figure 2) differed between soils ($P < 0.05$) but remained largely unaffected by soil cover or species diversity. However, legumes did increase [NH_4^+] ($P < 0.01$) and this effect originated essentially from the Ballantrae soil whereas no such effect was found in Flockhouse soil ($P = 0.001$ for soil×legume interaction). Soil [NO_3^-] (Figure 2) was ca. two orders of magnitude higher in unvegetated than in vegetated microcosms ($P < 0.001$) and decreased with increasing plant species diversity ($P < 0.05$). The presence of legumes highly significantly increased [NO_3^-] ($P < 0.001$); this effect was especially large in *Trifolium repens* monocultures, while positive effects of *T. subterraneum* and *T. pratense* (P~0.05) were found in mixtures. Even after accounting for effects of species and functional type diversity and for effects of single species (individual species contrasts, model 3 in Table 2), [NO_3^-] was highly significantly different between individual communities ($P < 0.001$ for mixture effect) and community effects on soil [NO_3^-] differed between soils ($P < 0.001$ for mixture×soil interactions), indicating complex interspecific interactions.

Nitrogen mineralisation

N mineralisation (Figure 3), measured under anaerobic conditions, differed significantly between soils ($P < 0.001$) and decreased with plant presence ($P < 0.05$), species diversity ($P < 0.05$) and functional type diversity ($P < 0.01$). Legumes increased N-mineralisation in Ballantrae but not in Flockhouse soils ($P < 0.05$ for legume×soil interaction). N mineralisation rates under aerobic conditions showed essentially the same effects (data not shown).

Potential nitrification

Effects on potential nitrification rates (Figure 3) roughly matched the observed effects on soil [NO_3^-]; potential nitrification rates were close to two orders of magnitude higher in microcosms lacking plant species ($P < 0.001$), and the effect of

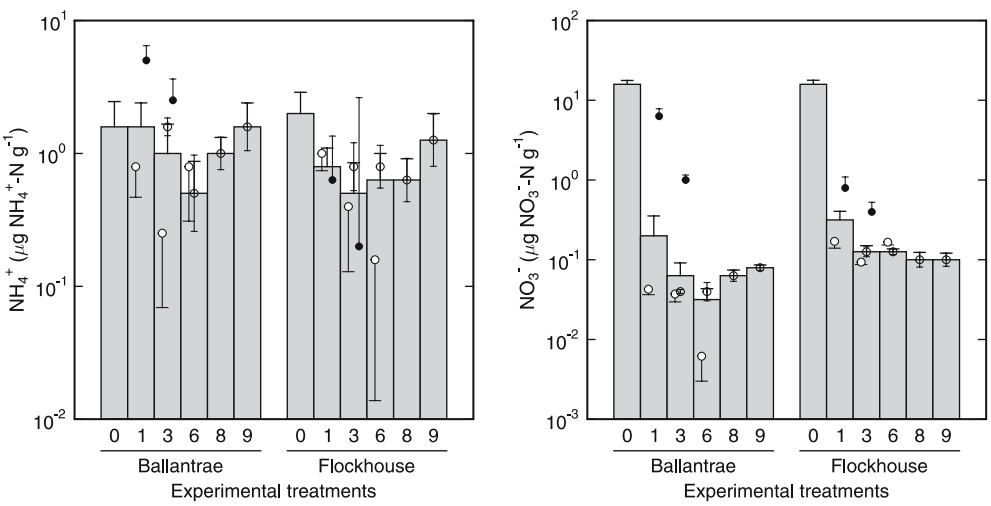

Figure 2. Soil NH_4^+ and NO_3^- concentrations as function of soil type, species richness, and composition within each level of species richness (means ± s.e.; black symbols, upward error bars: legumes only; white symbols, downward error bars: non-legumes only; open circles with bidirectional error bars: legumes and non-legumes mixed).

Table 4. Soil moisture and trace gas exchange rates (CH_4 and N_2O) as a function of soil type and date. Data are averages over all species richness levels (means s.e.)

Soil	Date	Soil moisture (g H_2O cm^{-3})	N_2O release (μmol m^{-2} d^{-1})	CH_4 uptake (μmol m^{-2} d^{-1})
Ballantrae	Dec 2001	0.28 ± 0.01	1.48 ± 0.19	55.12 ± 2.77
	Jan 2002	0.23 ± 0.01	0.61 ± 1.29	53.91 ± 1.49
	Mar 2002	0.31 ± 0.01	1.29 ± 1.15	52.99 ± 1.73
	May 2002	0.22 ± 0.01	2.00 ± 1.23	45.73 ± 1.05
Flockhouse	Dec 2001	0.27 ± 0.01	3.47 ± 1.17	9.84 ± 1.69
	Jan 2002	0.29 ± 0.01	2.37 ± 1.24	5.04 ± 0.45
	Mar 2002	0.29 ± 0.01	2.76 ± 1.17	7.72 ± 1.37
	May 2002	0.20 ± 0.01	2.20 ± 1.21	4.68 ± 0.405

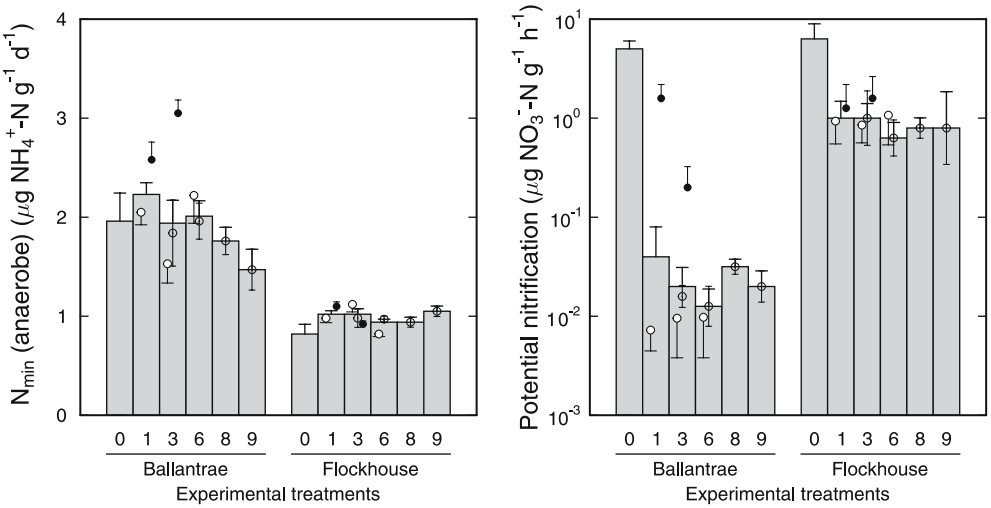

Figure 3. Nitrogen mineralisation (N_{min}; anaerobic soil incubations) and potential nitrification rates as function of soil type, species richness, and plant community composition (means ± s.e.; black symbols, upward error bars: legumes only; white symbols, downward error bars: non-legumes only; open circles with bidirectional error bars: legumes and non-legumes mixed).

soil cover was larger in Ballantrae soil ($P < 0.05$ for cover×soil interaction). In contrast to the effects on [NO_3^-], potential nitrification rates did not significantly depend on overall species or functional type diversity. However, it increased in the presence of legumes ($P < 0.001$) and this effect was greater in Ballantrae soils ($P < 0.001$ for legume×soil interaction). There was a significant interactive effect of soil type×forb presence on potential nitrification rates ($P < 0.05$); forbs reduced nitrification rates for the Ballantrae soils but not for the Flockhouse soils. As for effects on NO_3^-, there remained a highly significant difference between communities differing in composition even after accounting for species and functional type richness and the effect of presence of a single species or functional type ($P < 0.001$). There were significant interspecific interactions on potential nitrification rates which depended on soil type ($P < 0.01$ for mixture×soil interaction).

Soil trace gas exchange

N_2O emissions (Figure 4) were higher from the Flockhouse than from the Ballantrae soil ($P < 0.001$) and were significantly reduced in the presence of plants ($P < 0.001$). More diverse plant communities reduced N_2O emissions ($P < 0.01$ for species and functional type richness) from both soils; this effect was mainly driven by the higher emissions in monocultures whereas the micro-

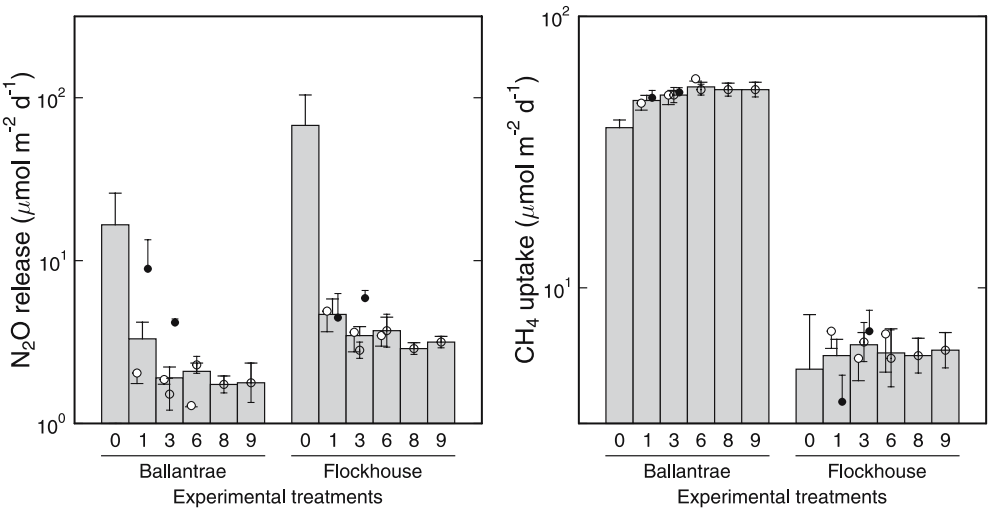

Figure 4. Net ecosystem CH_4 and N_2O exchange as function of soil type, species richness, and plant community composition (means ± s.e.; black symbols, upward error bars: legumes only; white symbols, downward error bars: non-legumes only; open circles with bidirectional error bars: legumes and non-legumes mixed).

cosms with six to nine species showed no or only little differences. The presence of legumes increased N_2O emissions ($P < 0.01$) and this effect was larger in Flockhouse soil ($P < 0.01$). The presence of non-legume forbs decreased N_2O emissions ($P < 0.001$).

Soils were sinks for CH_4 at all times (Figure 4); CH_4 uptake in Ballantrae soil was one order of magnitude higher than in Flockhouse soils ($P < 0.001$). Legume monocultures showed lower CH_4 uptake than non-legume monoculture ($P < 0.05$) in microcosms with Flockhouse soils, but no such effect was found in Ballantrae soils or in communities with higher diversity. However, a significant mixture×soil interaction ($P < 0.01$) indicated that interspecific interactions were important and that effects of species depended on soil type.

Relative importance of soil moisture and nitrate in controlling trace gas exchange

Since both soil moisture and NO_3^- availability are relatively direct drivers of deitrifier and possibly also methanotroph activity, we explored their relative importance by modelling N_2O emission rates as a function of soil type, soil moisture and soil NO_3^-. In these multiple regressions, all factors were highly significant regardless of their order; however, soil NO_3^- always explained much more variance than soil moisture, suggesting that it was a much more important determinant of these processes. For example, NO_3^- explained three times more variance than H_2O when fitted after soil moisture, and nine times more variance when fitted before soil moisture.

This finding was corroborated by model selection through stepwise multiple regression. The full model included soil type, soil cover (i.e., whether there were plants in microcosms), species richness, soil NO_3^-, soil NH_4^+, potential nitrification, and N-mineralisation rates (under aerobic and anaerobic conditions); the best submodel explained 65% of total variance and contained the factors soil type, NO_3^-, NH_4^+ and soil moisture; soil NO_3^- explained 2.5 to 15 times more variance than soil H_2O, depending on the relative order of factors.

Overall, this provides correlative evidence that both soil moisture and soil NO_3^- drove N_2O emission rates, but that soil NO_3^- was more important. When an additional factor classifying plant communities into legume-only, non-legume only, and mixed legume-non legume communities was introduced, this factor explained slightly more variance than NO_3^- and H_2O together; NO_3^- explained ≈1/3 less variance under these conditions than H_2O, but still remained

important. This indicates that NO_3^- levels were primarily driven by legume presence in communities, and that this was the main determinant of N_2O emissions.

A similar analysis for CH_4 exchange indicated that soil moisture, over the range which occurred during this study, did not affect CH_4 oxidation rates; the only significant factors remaining in the multiple regression model were soil type, soil cover, soil NO_3^- and potential nitrification rates. Potential nitrification rates explained a small but significant amount of total data variance (5–6% of the variance not explained by soil type; $P < 0.05$).

CH_4 oxidation in other field biodiversity studies

Soils from the calcareous grassland biodiversity study (Niklaus et al., 2001b) did not show any methanotrophic activity. This was confirmed by analysis of a fresh sample from the field site. Samples from the Swiss subexperiment of the BIODEPTH biodiversity study showed measurable methanotrophic activity, but results were equivocal (Figure 5): CH_4 oxidation rate constants decreased with diversity in the undisturbed part of the experiment, but increased in the subplots subjected to simulated trampling by grazers ($P < 0.01$ for diversity×trampling).

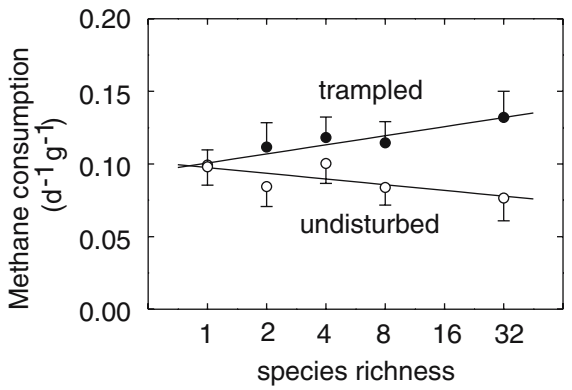

Figure 5. First-order rate constant (means ± s.e.) for CH_4 oxidation in the Swiss subexperiment of the BIODEPTH study. The study included a five levels of species richness and a factorial disturbance treatment ('trampling') simulating disturbance by ungulates. The trampling×richness interaction is significant at $P < 0.01$.

Discussion

Our results demonstrate that manipulations of plant species richness and community composition can affect the soil trace gas balance. Reductions in species richness resulted in less efficient capture of mineral N, increased soil NO_3^- concentrations and increased N_2O emissions.

This effect was predominantly driven by legume-only communities, which had dramatically increased NO_3^- levels, nitrifier populations and N_2O emissions. When legumes were grown together with non-legumes, this effect was much smaller or absent. Effects on CH_4 oxidation were less clear; plant community composition significantly affected CH_4 oxidation rates, but these effects could not be clearly related to changes in N cycling. Taken together, reducing species and particularly functional type diversity resulted in an increased emission of greenhouse gases from these model agro-ecosystems, providing evidence for a possible functional link between the composition and diversity of plant communities and global warming.

Diversity effects on soil nitrate and N_2O emissions

Effects of plant species diversity on soil NO_3^- have so far been detected in several studies. Ewel et al. (1991) reported increased soil [NO_3^-] under monoculture compared to more diverse tropical communities. Tilman et al. (1996), using grassland communities assembled by random selection of species from a common pool also found increased [NO_3^-] at low diversity. Hooper and Vitousek (1998) manipulated plant functional type richness and reported increased [NO_3^-] at low functional type diversity. Niklaus et al. (2001a) studied calcareous grassland communities for 5 years and found persistently increased soil [NO_3^-] in 5 species compared to 12 and 31 species communities. Scherer-Lorenzen et al. (2003) also reported increasing soil [NO_3^-] at reduced plant species and functional type diversity.

What are the mechanisms that can potentially lead to increased [NO_3^-] at low diversity?

First, if high diversity communities are more productive, this will be associated with higher uptake of soil inorganic N by plants and thus may result in lower soil NH_4^+ and NO_3^- pools.

This is to be expected under non-N-limited conditions because NH_4^+ from mineralisation or fertilization, which is not taken up by plants or immobilised by microbes will be oxidised by nitrifiers and result in elevated soil [NO_3^-].

Under N-limited conditions, however, net N-mineralisation approximately matches plant N uptake, and soil [NO_3^-] is the result of a dynamic equilibrium between mineralisation, nitrification and uptake of NH_4^+ and NO_3^- by plants. Low diversity communities may show spatially more uneven N capture than high diversity communities. This could lead to the local accumulation of NH_4^+, increased nitrification rates and increased [NO_3^-], even when total plant N uptake does not differ between diversity levels on a seasonal basis. Another possibility is that N mineralisation is not synchronised with plant N uptake, so that soil inorganic N temporally accumulates in soils – such a phenomenon could be less important at high diversity due to complementarity in the phenology of plant species.

Lower soil [NO^-_3] in high diversity communities might also be the result of an increased probability of including species which are particularly efficient in drawing down soil [NH_4^+] and [NO_3^-]; plant species differ in inorganic N root uptake kinetics so that the presence of individual species can affect steady-state concentrations of NH_4^+ and NO_3^-.

Finally, plants could interact with nitrifying microbes by mechanisms other than competition for NH_4^+. Plants can have species-specific effects on soil microbial communities, and plant-derived compounds that inhibit nitrification have been identified (e.g., tannins; Rice and Pancholy, 1973, 1974). The regulatory role of allelopathic inhibition for nitrification, however, is controversial (Schlesinger, 1996). Nevertheless, if some species were particularly efficient in suppressing nitrification, these effects could be more important in more diverse communities because the likelihood to comprise of such species would be higher ('sampling effect'; Aarssen, 1997; Huston, 1997). We do not have any indication that such effects occurred in the present study, but the possibility of such a mechanism cannot be ruled out in general.

It is difficult to separate these potential mechanism in any particular experiment. However, in most published studies plant biomass and N pools strongly increased with diversity (Hooper and Vitousek, 1998; Scherer-Lorenzen et al., 2003; Tilman et al., 1996, and the present study) and soil inorganic N availability most likely did not limit plant growth of low diversity communities. It therefore appears likely that the primary cause of increased soil [NO_3^-] was that net N mineralisation exceeded plant demand at low diversity; under these conditions the excess NH_4^+ was oxidised by nitrifiers which resulted in increased [NO_3^-]. A notable exception is the study by Niklaus et al. (2001a) who found persistently increased soil [NO_3^-] over 5 years, with only small differences in plant N pools and no differences in measured N mineralisation rates, suggesting that some of the alternative mechanisms suggested are also involved.

It has been proposed that increased [NO_3^-] concentrations may not be relevant from an ecosystem functioning perspective since the extra [NO_3^-] is not necessarily lost through leaching. Indeed, Hooper and Vitousek (1998) did not find a systematic change in below-rooting zone [NO_3^-]. Similarly, Scherer-Lorenzen et al. (2003), using a hydrological model and semi-continuous suction-cup data, did not find evidence of greater annual NO_3^- leaching under low plant diversity, at least when legumes were absent. Our study shows, however, that N_2O produced through nitrification and denitrification is increasingly lost to the atmosphere under these conditions. This probably also applies to emissions of NO and N_2 and (which were not measured) demonstrates that increased [NO_3^-] can reduce N retention, even in the absence of significant leaching.

Diversity effects on soil CH_4 oxidation

Microbial oxidation of CH_4 tended to be slightly lower under low than under high diversity, but these differences were not statistically significant. However, legumes significantly reduced the soil CH_4 sink on the more fertile soil, and plant species composition did significantly affect the soil CH_4 sink.

The mechanisms by which soil methanotrophic activity interacts with the soil N cycle are poorly understood. NH_4^+ competitively binds to the enzyme catalysing the first step of CH_4 oxidation (MMO: methane mono-oxygenase), thus reducing CH_4 oxidation rates (Carlsen et al., 1991). This

mechanism has been investigated in laboratory conditions and was apparently corroborated by the observation that NH_4^+ fertiliser suppressed soil CH_4 uptake in the field (Mosier et al., 1991) while NO_3^- fertilisation did not (Hütsch et al., 1993). However, competitive binding of NH_4^+ to MMO, often recognised as the key mechanism (Dunfield and Knowles, 1995), is probably insufficient to explain the suppression of methanotrophy in the field, because effects often last long after [NH_4^+] has returned to pre-application levels. Other possible mechanisms include competitive interactions between methanotrophs and soil microbes involved in the N cycle, e.g., ammonia oxidisers, starvation responses, and other effects on methanotroph population dynamics (Powlson et al., 1997; Schimel, 2000). Furthermore, the observation that NH_4^+ sometimes stimulates CH_4 oxidation instead of inhibiting it, and that sometimes soil [NH_4^+] and CH_4 oxidation are positively correlated, has recently cast doubts on the hypothesis of enzymatic inhibition as main control mechanism (Bodelier and Laanbroek, 2004; Bodelier et al., 2000). The conclusion is that a general mechanistic concept of nutrient interactions with CH_4 oxidation in intact soil has yet to be developed.

Most studies reporting large inhibitory effects of NH_4^+ have involved the use of NH_4^+-fertiliser. Application of mineral fertiliser is likely to result in a high peak [NH_4^+] when compared to plant-mediated alterations of a soil's intrinsic mineralisation processes. In our study, plant diversity-effects on N cycling were small and did probably not lead to high peak [NH_4^+], which may explain the absence of stronger effects on the soil CH_4 sink. Alternatively, CH_4 oxidation may have been co-limited by other effects, e.g., soil diffusive conductance (Del Grosso et al., 2000; Smith et al., 2003). However, it is also possible that responses of methanotrophs are inherently slow (Prieme et al., 1997) and that larger effects would have been found if the experiment had run longer.

For this reason, we also analysed soil methanotrophic activity in two field biodiversity studies which both ran for 5 years. Soils in the first (Niklaus et al., 2001b) did not show any measurable CH_4 uptake, which is surprising in the light that this system was N-poor and that soils were well aerated. Responses in the second study (Hector et al., 1999) were equivocal; while significant effects were found, their direction reversed when plots were mechanically disturbed ('trampling' treatment). No data regarding nitrogen cycling is available from the disturbed subplots in this latter study; we can therefore only speculate that effects of the trampling treatment on soil diffusive conductance and on plant biomass (losses were higher at high diversity; cf. Pfisterer and Schmid, 2002) may have been involved in this reversal of CH_4 oxidation-response to species richness. Nevertheless, this example not only demonstrates that biodiversity can affect CH_4 fluxes but also the complexity of ecological interactions to consider if findings ought to be generalisable. It also appears necessary to investigate effects under a wide range of conditions.

Compositional vs. species richness effects

Our results suggest that compositional effects, i.e., the specific nature and combination of species ('mixture'-term in ANOVA model), were more important than the number of species or functional types *per se*. Of particular importance was the presence of legumes. Legumes play a keystone function in terrestrial N cycling because of their ability to symbiotically fix atmospheric N_2. Intercropping studies have shown that the presence of legumes can increase soil [NO_3^-] (Mallarino and Wedin, 1990; Ranells and Wagger, 1997), primarily due to losses of symbiotically fixed N through turnover of root and shoot tissue; legumes also are generally ineffective in capturing soil mineral N. Indeed, communities consisting of legumes only (1 or 3 species mixtures) showed the highest plant N pools, soil [NO_3^-], nitrification rates, and N_2O emissions. These effects were mitigated by the presence of non-legume species, primarily because these species drew down soil mineral N pools. Species and functional type richness are inherently related, and since the probability of communities to contain legumes only was increased in the 1 and 3 species cultures, a significant part of the observed species richness-effect is in fact due to legume-only communities.

However, other compositional effects were also important; this is evidenced by statistically significant effects of species mixture for all parameters tested, which explains more variance

than species richness *per se*. This was the case even after accounting for legume presence, functional type composition, or the presence of individual species. These effects were not simply due to individual species, but also due to their interactions. The presence of statistically significant mixture×soil type interactions shows that the underlying species mixtures performed differently depending on soil type. At the more aggregated level of species richness and functional type-diversity, however, effects were remarkably similar for both soils, despite differences in physical structure and nutritional status.

Our findings are in line with Hooper and Vitousek (1997) who reported effects of plant functional type diversity and composition in grassland communities. In their study, legumes dominated effects on soil [NO_3^-], while another functional group, early seasonal annuals, reduced soil [NO_3^-] to low levels when present. Similarly, Scherer-Lorenzen et al. (2003), studying grassland communities at the German site of the BIODEPTH experiment, found largely increased soil [NO_3^-] in the presence of legumes, and effects of species richness *per se* were small when compared to the variance explained by community composition.

Conclusions

Low diversity communities were less efficient in capturing soil mineral N, which resulted, especially when legumes were present, in increased nitrification rates and N_2O emissions from the ecosystem. Community composition also significantly affected soil CH_4 oxidation. Our study provides evidence that plant community diversity and composition can alter the trace gas balance of agro-ecosystems, suggesting a possible link between biodiversity and global warming.

Compositional effects have been found to be more important than effects of species richness *per se* also in previous studies of plant performance – our findings generalise this conclusion by extending its scope to the soil trace gas balance. We also show that composition strongly interacts with soil type, i.e., that specific plant mixtures perform vastly different depending on soil. However, at the aggregated level of species numbers *per se*, responses were remarkably similar. At this level, between mixture-differences average out and results become relatively robust, at least with the given pool of species and environmental conditions.

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